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Do saliva concentrations predict plasma unbound theophylline concentrations? A problem re-examined

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1 On the assumption that plasma unbound drug concentrations are therapeutically active, the value of saliva concentrations in predicting plasma unbound theophylline concentrations was investigated in 25 ambulatory adults (aged 27 to 84 years) receiving theophylline (225–1350 mg aminophylline daily) for asthma or chronic bronchitis.

2 Plasma samples from all patients were ultrafiltered, and the plasma unbound theophylline (F) concentrations were compared with the corresponding total plasma (P), citric acid stimulated saliva (S) and non-stimulated saliva (Ns) theophylline concentrations.

3 Plasma unbound theophylline concentrations correlated significantly with P ($r = 0.97$) and S ($r = 0.973$), but less well with Ns ($r = 0.883$), emphasising the benefit of saliva stimulation.

4 The ability of S to predict F theophylline concentrations was assessed using the mean ratio of 0.7297. In 92% of the patients, predicted F concentrations were within ± 1 μg/ml of the measured concentrations. Similarly, using the mean F/P ratio of 0.418, predicted P were within ± 1 μg/ml of obtained P in 84% patients, and using the mean S/P ratio of 0.568, predicted P were within ± 1 μg/ml of obtained P in 81%.

5 An accuracy of ± 1 μg/ml in estimating F from S concentrations would be sufficient to indicate appropriate dose adjustments, and we therefore advocate the use of stimulated saliva samples for routine monitoring of theophylline therapy.

Keywords theophylline saliva concentration plasma unbound concentration

Introduction

Theophylline is used in this hospital primarily in the treatment of asthma, bronchitis and neonatal apnoea. In adults, theophylline plasma concentrations have been shown to correlate linearly with bronchodilator effect within the concentration range of 10–20 μg/ml (Mitenko *et al.*, 1973; Levy & Koysooko, 1975; Barclay *et al.*, 1981). Theophylline elimination may be altered by smoking, disease, old age (Antal *et al.*, 1981; Paterson & Ilett, 1981), childhood (Ellis *et al.*, 1976) diet, and environmental insults (Decourt *et al.*, 1982).

In some individuals, small dose increments can result in adverse reactions with little or no improvement in lung function (Ogilvie, 1981), and the risk of hepatic enzyme saturation which results in disproportionately high plasma concentrations for small dose increments (Weinberger & Ginchansky, 1977; Sarrazin *et al.*, 1980), further emphasizes the need for routine monitoring of theophylline therapy (Wyatt *et al.*, 1978).

Frequent blood sampling for dosage evaluation is often necessary in growing children and

premature neonates. However, the problem of obtaining even small, non-haemolysed blood samples from neonates, and the desire to avoid emotional trauma associated with venepuncture in children (and some adults!) prompted us to investigate the value of saliva as a non-invasive index of therapeutically active theophylline. The literature contains conflicting reports of the suitability of saliva for monitoring theophylline therapy (Koysooko *et al.*, 1974; Boobis *et al.*, 1979; Culig *et al.*, 1982; Goldsworthy *et al.*, 1981) but in none of these studies have saliva theophylline concentrations been directly related to the therapeutically active plasma unbound theophylline concentrations.

We have therefore measured theophylline concentrations in paired saliva, plasma total and plasma unbound samples from out-patients receiving theophylline.

Methods

Subjects Twenty-five adults (14 male), aged 27 to 84 years, receiving 225–1350 mg aminophylline (Phyllocontin SR) daily, were studied. Some patients also received intermittent prednisolone, salbutamol, terbutaline, sodium cromoglycate and a variety of high ceiling diuretics and cardiotonics. Two children (aged 3 and 11 years) receiving 240 and 675 mg/day and two neonates aged 6 and 8 days, receiving 8 mg/day aminophylline i.v. were also studied.

Sample collections In all adult patients venous blood (26 ml) was taken, heparinised and the plasma separated immediately. Approximately 0.5 ml of blood was obtained from the neonates by heel prick, and 1–2 ml of venous blood was taken from the children. Each adult was asked to spit into a container until 0.5–1.0 ml of mixed saliva was collected, and in all patients a sample of mixed saliva was collected using citric acid (approximately 5 mg) stimulation as previously described (Reynolds *et al.*, 1976). Citric acid stimulated saliva was easily obtained from the oral cavity of the neonates using gentle suction with a syringe. Where possible, the pH of the saliva sample was measured immediately using a Digital pH/mV and temperature meter (Electronic Instruments Limited).

Protein binding estimations Plasma containing theophylline was ultrafiltrated under reduced pressure through dialysis tubing (Visking size 1–8/32 in; molecular weight cut off point 14,000) at room temperature (22 ± 1°C) and pH 7.4, as described eleswhere (Reynolds *et al.*, 1976). There was negligible non-specific binding to the dialysis tubing under these conditions of temperature and pH. Citric acid stimulated saliva was spiked with theophylline (10 μg/ml) and ultrafiltrated for protein binding estimations. Salicylsulphonic acid (25%) was used to test the filtrates for the presence of protein.

Analysis was carried out using a homogenous enzyme immunoassay, EMIT (Syva Company) in which the rate of conversion of NAD to NADH is measured spectrophotometrically and is related to the concentration of theophylline in the sample.

Full ethical permission and patients' consent was obtained before embarking on this study.

Results

The reproducibility of the assay as estimated by coefficient of variation is for plasma and saliva samples approximately 3%, and for filtrates, less than 2%. Although a standard curve is stable in the microprocessor for at least 10 days, full calibrations were carried out at each analysis.

Concentrations of theophylline in plasma obtained by finger prick from one volunteer correlated closely with venous plasma theophylline concentrations (r = 0.958, mean plasma/finger prick ratio = 1.126).

The relationship between theophylline concentrations in plasma (P), stimulated saliva (S), and that free in plasma water (F), are presented in Figures 1 and 2. All correlations are high (r = 0.97, Table 1). The mean theophylline plasma protein binding was 57.7 ± 5.06% in males, and 58.8 ± 5.1% in females (overall mean = 58.2 ± 5.09%) and a very weak correlation was found between age and protein binding (r = 0.411).

Fifteen patients were unable to produce a sample of non-stimulated saliva (Ns), and in three the theophylline concentration in the Ns sample was immeasurably high whereas that in the paired stimulated sample was approximately 0.57 of the concentration in the corresponding plasma sample. Excluding the immeasurably high Ns values, the relationship between Ns and F was still less good (r = 0.883, slope Ns/F = 1.491) than that between S and F (Table 1). In two patients however, all saliva samples were contaminated with drug in spite of careful questioning about dental hygiene. These patients both had false teeth and mouth washing did not remove all traces of contaminant from the mouth! The mean pH of Ns samples was 6.51 ± 0.5 and that of the S samples was 3.712 ± 1.05, lower due to the presence of citric acid (unpublished observation). No relationship between non-stimulated saliva pH and the logarithm of

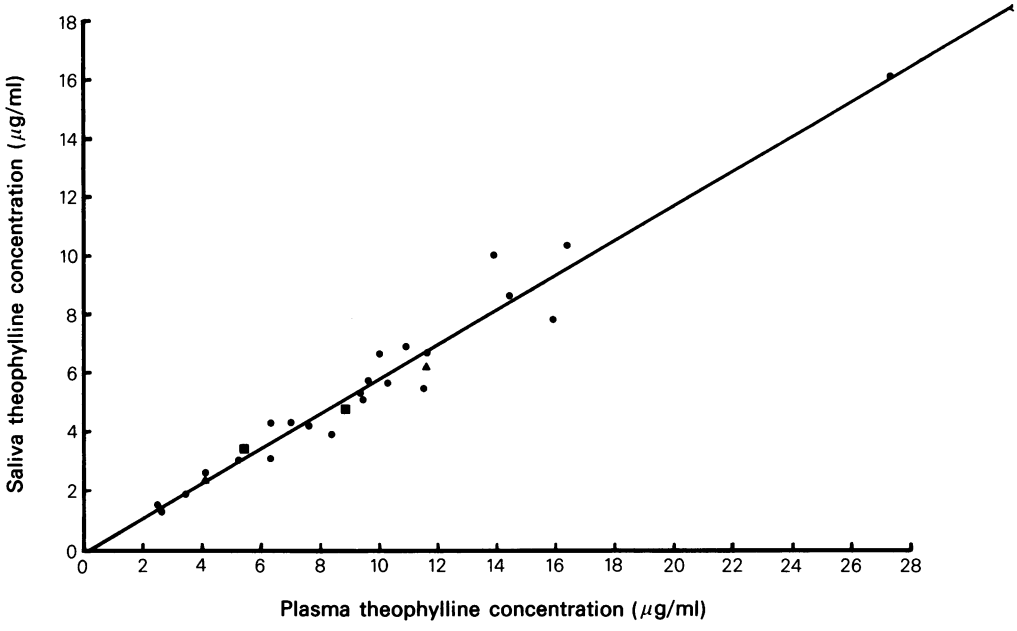

Figure 1 Correlation between saliva and plasma theophylline concentrations in 23 adults (●), 2 children (▲), and 2 neonates (■).

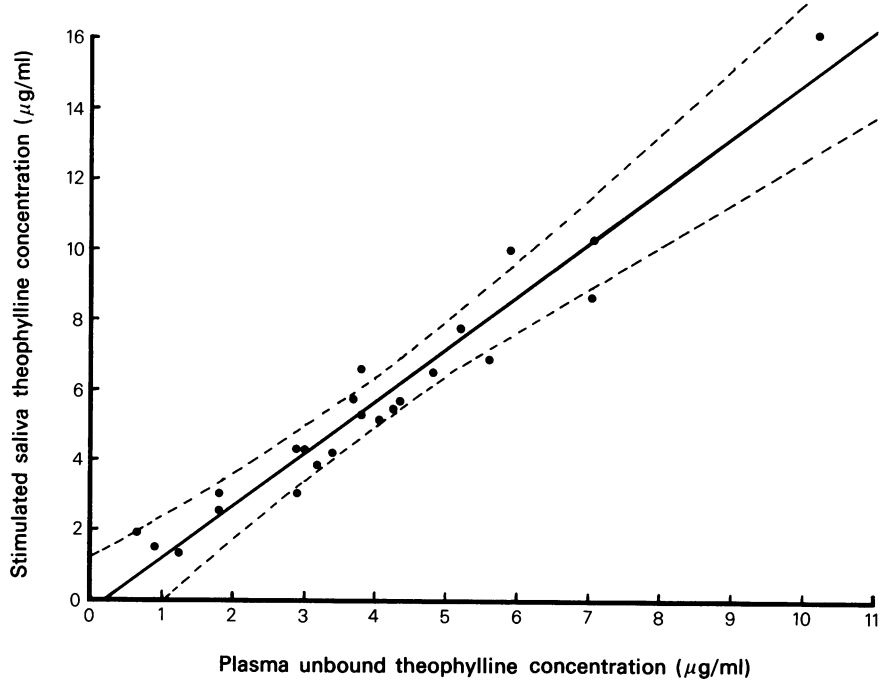

Figure 2 Correlation between saliva and plasma unbound theophylline concentrations in 23 adults.

Table 1 The relationship between S, P and F theophylline for all patients.

	S vs *P* (y) (x)	*F* vs *P* (y) (x)	*S* vs *F* (y) (x)
n	27	25	23
Mean ratio y/x	0.568	0.418	1.414
s.d.	0.078	0.049	0.204
r	0.977	0.97	0.973
Slope	0.591	0.382	1.497
intercept	–0.111	0.31	–0.324
Mean x	9.4	9.56	4.0
Range	(2.6–27.3)	(2.6–27.3)	(0.9–10.2)
Mean y	5.44	3.96	5.67
Range	(1.3–16.1)	(0.9–10.2)	(1.3–16.1)

either the F/Ns ratio ($r = 0.258$) or the Ns/P ratio ($r = -0.288$) was found however, and there was no binding to saliva proteins.

Discussion

The use of saliva as a means of drug monitoring has increased steadily during the last 5 years, largely due to an improvement in assay techniques and equipment. In this laboratory saliva is used routinely to monitor anticonvulsant therapy (Knott & Reynolds, 1983a) either in order to monitor free drug concentrations (Reynolds *et al.*, 1976; Knott *et al.*, 1982) or for humane reasons (Knott & Reynolds, 1982).

Citric acid stimulated saliva theophylline concentrations correlated closely with plasma theophylline concentrations in all patients ($r = 0.977$) with a mean (± s.d.) S/P ratio of 0.568 ± 0.008 and the same relationship between saliva and plasma was found in subjects of all ages (Figure 1). Although other laboratories obtained similar ratios in children (Levy *et al.*, 1974; Eney & Goldstein, 1976; Galant *et al.*, 1977; Kelly *et al.*, 1981; Goldsworthy *et al.*, 1981), Khanna and colleagues (1980) found the saliva theophylline concentrations approximated to 0.76–0.8 of plasma theophylline concentrations in neonates. Further studies are clearly needed to confirm the S/P ratio in neonates found in this study.

A significant number of patients were unable to produce sufficient saliva for drug analysis without first being given citric acid, because their mouths were dry. Some non-stimulated saliva samples were of high viscosity making sample aspiration into the pipetter diluter difficult, and initial absorbance values high. In cases where saliva samples contained suspended oral debris, centrifugation prior to analysis frequently improved the precision of the assay. Even after elimination of these potential sources of assay error there was a large variation in the relationship between non-stimulated saliva and plasma

Table 2 Mean S/P ratios obtained in other laboratories.

A/C	*S/P ratio*	(r)	*% Bound*	*Separation technique*	*Type of saliva*	*Assay*	*Authors*
A	NA	(0.91)	43.4	Eq. D	S	EMIT	Boobis *et al.* (1979)
C	NA	(0.94)			Ns	& g.l.c.	
A	0.46	(0.81)			S	EMIT	Culig *et al.* (1982)
C	0.5	(0.89)			P	EMIT	Goldsworthy *et al.* (1981)
A	0.52		59.0	Eq. D	S	Spec.	Koysooko *et al.* (1974)
A	0.513	(0.93)			S	RIA	Plasvic *et al.* (1981)
C	0.577	(0.95)			S	Spec.	Levy *et al.* (1974)
C	0.67	(0.99)			P	h.p.l.c.	Galant *et al.* (1977)
A	0.625	(0.85)			P	Spec.	Hendeles *et al.* (1977)
C	0.71	(0.75)			Ns	EMIT	Lena *et al.* (1980)
C	0.926	(0.7)			Ns	h.p.l.c.	Khanna *et al.* (1980)
C	0.658	(0.8)			P	h.p.l.c.	Kelly *et al.* (1981)
C	0.65	(0.972)			S	g.l.c.	Eney & Goldstein (1976)

Key: A = Adults, C = Children
Types of saliva:
S = Stimulated mixed saliva either by mastication or gustatory stimulation.
Ns = Non-stimulated mixed saliva
P = Parotid saliva
Methods:
Spec = Spectrophotometry
g.l.c. = Gas liquid chromatography
EMIT = Enzyme linked immunoassay
RIA = Radio-immunoassay
h.p.l.c. = High pressure liquid chromatography

unbound theophylline concentrations. Stimulating salivation maximises flow rate and appears to minimise the S/P variation. The different types of saliva collected by different laboratories may partly explain the wide interlaboratory variation in S/P ratios (Table 2) while, probably because saliva collection is standardised *within* a centre, intralaboratory S/P variations are lower.

Several authors have reported that the secretion of theophylline into saliva is not influenced by saliva pH and flow rates (Levy *et al.*, 1974; Culig *et al.*, 1982). In this study, no correlation was obtained between non-stimulated saliva pH and either the logarithm of the Ns/P ratio, or the F/Ns ratio, as has been reported for pH and flow dependent drugs (McAuliffe *et al.*, 1977). We were also unable to demonstrate theophylline binding to pooled stimulated saliva, making it unlikely that theophylline concentrations in saliva are influenced directly by variations in its protein content.

The relationship between F and P is linear over the range studied, and F correlates well with P ($r = 0.97$). The mean plasma protein binding in these patients of 58.2 ± 5.09%, is similar in males (57.7 ± 5.06%) and females (58.8 ± 5.1%). These findings are consistent with those of other authors using ultrafiltration (Antal *et al.*, 1981; Aslaksen *et al.*, 1981; Yurchak & Jusko, 1976; Piafsky *et al.*, 1980; Mangione *et al.*, 1978) but somewhat higher than those obtained by equilibrium dialysis at 37°C (Buss *et al.*, 1983; Brǿrs *et al.*, 1983) showing that theophylline binding is temperature dependent, as well as pH dependent (Vallner *et al.*, 1979). There also appears to be a very weak correlation between age and free fraction ($r = 0.411$) such that theophylline plasma protein binding decreases with advancing years, as reported for other drugs (Wallace *et al.*, 1976; Patterson *et al.*, 1982). Clinically, this is often associated with a decreasing dose requirement (Crooks *et al.*, 1976).

Although the correlation between stimulated saliva and plasma unbound theophylline concentrations was high ($r = 0.973$) and the confidence zone narrow (Figure 2), the ability of saliva to predict free plasma theophylline was examined using the mean F/S ratio of 0.7297. In 92% of the patients, predicted plasma free theophylline concentrations were within ±1 μg/ml of the obtained concentration. This was slightly better than that found when the mean F/P or S/P ratios (Table 1) were used to predict plasma theophylline concentrations. (Predicted concentrations were within ±1 μg/ml of that obtained in 84% of patients using F/P, and 81% using S/P). The mean theophylline concentration in saliva was 5.6 μg/ml, in plasma was 9.56 μg/ml, and free in plasma water was 4 μg/ml. Over- or under-estimations of ±1 μg/ml would be insufficient to make dose modifications inaccurate.

We therefore conclude that theophylline therapy may be routinely monitored using stimulated saliva samples when the usual precautions are taken to ensure good dental hygiene (Knott & Reynolds, 1983b). Indeed it would be unethical either to check a level routinely, or to assess a child's compliance using plasma when an accurate, non-invasive alternative is available.

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